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<110> THE GOVERNMENT OF THE UNITED STATES OF AMERICA AS
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SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH

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<120> IN-VIVO CYTOTOXIC ACTIVITIES OF RECOMBINANT IMMUNOTOXIN 8H9
(FV)-PE38 AGAINST BREAST CANCER, OSTEOCARCINOMA AND NEUROBLASTOMA

<130> 4239-67287-05

<150> PCT/US03/038227

<151> 2003-12-01

<150> US 60/430,305

<151> 2002-12-02

<160> 17

<170> PatentIn version 3.2

<210> 1

<211> 731

<212> DNA

<213> Mus musculus

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cctgaacagg gacttgagtg gattggatgg attttcctg gagatggtag tactcaatac 180
aatgagaagt tcaaggccaa ggccacactg actacagaca catcctccag cacagcctac 240
atgcagctca gcaggctgac atctgaggac tctgctgtct atttctgtgc aagacagact 300
acggctacct ggttgctta ctggggccaa gggaccacgg tcaccgtctc ctcagatgga 360
ggcggttcag gcggaggtgg ctctggcggt ggcggatcgg acatcgagct cactcgtct 420
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agtattagcg actacttaca ctggtagccaa caaaaatcac atgagtctcc aaggcttctc 540
atcaaatatg cttcccaatc catctctggg atcccccca ggttcagttgg cagtggatca 600
gggtcagatt tcactctcag tatcaacagt gtggAACCTG aagatgttgg agtgttattac 660
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<210> 2
<211> 243
<212> PRT
<213> Mus musculus

<400> 2

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Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Asp Ile Asn Trp Val Arg Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Phe Pro Gly Asp Gly Ser Thr Gln Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Thr Asp Thr Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Arg Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gln Thr Thr Ala Thr Trp Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser Asp Gly Gly Ser Gly Gly Gly Ser
115 120 125

Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Thr Thr Leu
130 135 140

Ser Val Thr Pro Gly Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln
145 150 155 160

Ser Ile Ser Asp Tyr Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser
165 170 175

Pro Arg Leu Leu Ile Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro
180 185 190

Ser Arg Phe Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile

195

200

205

Asn Ser Val Glu Pro Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly
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His Ser Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
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Gln Ala Ala

<210> 3
<211> 243
<212> PRT
<213> Mus musculus

<400> 3

Gln Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Glu Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Asp Ile Asn Trp Val Arg Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Phe Pro Gly Asp Gly Ser Thr Gln Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Thr Asp Thr Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Arg Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gln Thr Thr Ala Thr Trp Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser Asp Gly Gly Ser Gly Gly Gly Ser
115 120 125

Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Thr Thr Leu
130 135 140

Ser Val Thr Pro Gly Asp Gln Val Ser Leu Ser Cys Arg Ala Ser Gln
145 150 155 160

Ser Ile Ser Asp Tyr Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser
165 170 175

Pro Gln Leu Leu Ile Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro
180 185 190

Ser Arg Phe Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile
195 200 205

Asn Ser Val Glu Pro Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly
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His Ser Phe Pro Leu Thr Phe Gly Ala Gly Thr Glu Leu Glu Leu Glu
225 230 235 240

Gln Ala Ala

<210> 4
<211> 354
<212> DNA
<213> Mus musculus

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cctgaacagg gacttgagtg gattggatgg attttcctg gagatggtag tactcaatac 180
aatgagaagt tcaaggggcaa ggccacactg actacagaca catcctccag cacagcctac 240
atgcagctca gcaggctgac atctgaggac tctgctgtct atttctgtgc aagacagact 300
acggctacct ggtttgctta ctggggccaa gggaccacgg tcaccgtctc ctca 354

<210> 5
<211> 321
<212> DNA
<213> Mus musculus

<400> 5
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catgagtctc caaggcttct catcaaatat gcttcccaat ccatctctgg gatcccctcc 180
agttcagtg gcagtggatc agggtcagat ttcactctca gtatcaacag tgtggaacct 240
gaagatgtt gagtgttatta ctgtcaaaat ggtcacagct ttccgctcac gttcggtgct 300
gggaccaagc tggagctgaa a 321

<210> 6
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Linker used to produce an 8H9 scFV.

<400> 6
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<210> 7
<211> 118
<212> PRT
<213> Mus musculus

<400> 7

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
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Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Asp Ile Asn Trp Val Arg Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Phe Pro Gly Asp Gly Ser Thr Gln Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Asp Thr Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Arg Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gln Thr Thr Ala Thr Trp Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

<210> 8
<211> 107
<212> PRT
<213> Mus musculus

<400> 8

Asp Ile Glu Leu Thr Gln Ser Pro Thr Thr Leu Ser Val Thr Pro Gly
1 5 10 15

Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr
20 25 30

Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
35 40 45

Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
65 70 75 80

Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu
85 90 95

Thr Phe Gly Gly Thr Lys Leu Glu Leu Lys
100 105

<210> 9
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker used to produce an 8H9 scFV.

<400> 9

Asp Gly Gly Gly Ser Gly Gly Ser Gly Gly Gly Ser
1 5 10 15

<210> 10
<211> 613
<212> PRT
<213> Pseudomonas aeruginosa

<400> 10

Ala Glu Glu Ala Phe Asp Leu Trp Asn Glu Cys Ala Lys Ala Cys Val
1 5 10 15

Leu Asp Leu Lys Asp Gly Val Arg Ser Ser Arg Met Ser Val Asp Pro
20 25 30

Ala Ile Ala Asp Thr Asn Gly Gln Gly Val Leu His Tyr Ser Met Val
35 40 45

Leu Glu Gly Gly Asn Asp Ala Leu Lys Leu Ala Ile Asp Asn Ala Leu
50 55 60

Ser Ile Thr Ser Asp Gly Leu Thr Ile Arg Leu Glu Gly Gly Val Glu
65 70 75 80

Pro Asn Lys Pro Val Arg Tyr Ser Tyr Thr Arg Gln Ala Arg Gly Ser
85 90 95

Trp Ser Leu Asn Trp Leu Val Pro Ile Gly His Glu Lys Pro Ser Asn
100 105 110

Ile Lys Val Phe Ile His Glu Leu Asn Ala Gly Asn Gln Leu Ser His
115 120 125

Met Ser Pro Ile Tyr Thr Ile Glu Met Gly Asp Glu Leu Leu Ala Lys
130 135 140

Leu Ala Arg Asp Ala Thr Phe Phe Val Arg Ala His Glu Ser Asn Glu
145 150 155 160

Met Gln Pro Thr Leu Ala Ile Ser His Ala Gly Val Ser Val Val Met
165 170 175

Ala Gln Thr Gln Pro Arg Arg Glu Lys Arg Trp Ser Glu Trp Ala Ser
180 185 190

Gly Lys Val Leu Cys Leu Leu Asp Pro Leu Asp Gly Val Tyr Asn Tyr
195 200 205

Leu Ala Gln Gln Arg Cys Asn Leu Asp Asp Thr Trp Glu Gly Lys Ile
210 215 220

Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile Lys
225 230 235 240

Pro Thr Val Ile Ser His Arg Leu His Phe Pro Glu Gly Gly Ser Leu
245 250 255

Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro Leu Glu Thr Phe
260 265 270

Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu Glu Gln Cys Gly
275 280 285

Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala Ala Arg Leu Ser
290 295 300

Trp Asn Gln Val Asp Gln Val Ile Arg Asn Ala Leu Ala Ser Pro Gly
305 310 315 320

Ser Gly Gly Asp Leu Gly Glu Ala Ile Arg Glu Gln Pro Glu Gln Ala
325 330 335

Arg Leu Ala Leu Thr Leu Ala Ala Ala Glu Ser Glu Arg Phe Val Arg
340 345 350

Gln Gly Thr Gly Asn Asp Glu Ala Gly Ala Ala Asn Ala Asp Val Val
355 360 365

Ser Leu Thr Cys Pro Val Ala Ala Gly Glu Cys Ala Gly Pro Ala Asp
370 375 380

Ser Gly Asp Ala Leu Leu Glu Arg Asn Tyr Pro Thr Gly Ala Glu Phe
385 390 395 400

Leu Gly Asp Gly Gly Asp Val Ser Phe Ser Thr Arg Gly Thr Gln Asn
405 410 415

Trp Thr Val Glu Arg Leu Leu Gln Ala His Arg Gln Leu Glu Glu Arg
420 425 430

Gly Tyr Val Phe Val Gly Tyr His Gly Thr Phe Leu Glu Ala Ala Gln
435 440 445

Ser Ile Val Phe Gly Gly Val Arg Ala Arg Ser Gln Asp Leu Asp Ala
450 455 460

Ile Trp Arg Gly Phe Tyr Ile Ala Gly Asp Pro Ala Leu Ala Tyr Gly
465 470 475 480

Tyr Ala Gln Asp Gln Glu Pro Asp Ala Arg Gly Arg Ile Arg Asn Gly
485 490 495

Ala Leu Leu Arg Val Tyr Val Pro Arg Ser Ser Leu Pro Gly Phe Tyr
500 505 510

Arg Thr Ser Leu Thr Leu Ala Ala Pro Glu Ala Ala Gly Glu Val Glu
515 520 525

Arg Leu Ile Gly His Pro Leu Pro Leu Arg Leu Asp Ala Ile Thr Gly
530 535 540

Pro Glu Glu Glu Gly Gly Arg Leu Glu Thr Ile Leu Gly Trp Pro Leu
545 550 555 560

Ala Glu Arg Thr Val Val Ile Pro Ser Ala Ile Pro Thr Asp Pro Arg
565 570 575

Asn Val Gly Gly Asp Leu Asp Pro Ser Ser Ile Pro Asp Lys Glu Gln
580 585 590

Ala Ile Ser Ala Leu Pro Asp Tyr Ala Ser Gln Pro Gly Lys Pro Pro
595 600 605

Arg Glu Asp Leu Lys
610

<210> 11
<211> 4
<212> PRT
<213> Pseudomonas aeruginosa

<400> 11

Lys Asp Glu Leu
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<210> 12

<211> 4
<212> PRT
<213> *Pseudomonas aeruginosa*

<400> 12

Arg Glu Asp Leu
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<210> 13
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer.

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<210> 14
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer.

<400> 14
agctgctgga tagtgcatat gcaggtccaa ctgcagcagt ctggggctga actg 54

<210> 15
<211> 93
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer.

<400> 15
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gcaagcttgt gaggagacgg tgaccgttgt ccc 93

<210> 16
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer.

<400> 16

tctggcggtg gccatatgga catcgagctc actcagtctc caaccacc

48

<210> 17
<211> 66
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer.

<400> 17
ctcgggagaa ttctatcatt tcagctccag cttggtccca caaccgaacg tgagcggaaa 60

gctgtg 66